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PRINCIPAL INVESTIGATOR: Thomas Ernst, Ph.D.

CONTRACTING ORGANIZATION: Harbor-University of California
Los Angeles Research and Education
Institute
Torrence, California 90502-2064

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Thomas Ernst, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Harbor-University of California Los Angeles Research and Education Institute
Torrence, California 90502-2064

E-Mail:

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2001 progress report for project # DAMD17-99-1-9210

"Brain Function, Structure, and Neurochemistry after Tamoxifen / Chemotherapy Assessed by Neuropsychologic Testing and 1H Magnetic Resonance Spectroscopy"

(1) INTRODUCTION

Loss of mental abilities represents a recognized threat to the quality of life of postmenopausal women with advancing age. Most recently, several reports have used a sensitive method (neuropsychological testing) to evaluate younger women with breast cancer after chemotherapy and hormonal modifying therapy (with tamoxifen), and found that a substantial percentage of these women had reduced mental abilities compared to women who were not treated with chemotherapy and hormonal modifying therapy. It also appears that these mental deficits are overlooked by the screening tests currently used in many large-scale breast cancer treatment and prevention studies, most likely because these simple screening tests become abnormal only when the brain is damaged to a moderate or severe degree. In the previous studies, most of the women with mental deficits obtained both chemotherapy and hormonal modifying therapy, so that it is unclear which of the two therapies caused the mental deficits. Furthermore, tens of millions of healthy women without breast cancer may soon obtain hormonal modifying therapy (with tamoxifen and possibly other drugs) to prevent future breast cancer; therefore, it is extremely important to know whether these drugs may cause injury to the brain and long-lasting problems with mental abilities. This study is designed to address these questions.

(2) BODY

Since the receipt of the funds in September 1999, we have made excellent progress towards accomplishing our goals. Altogether, we have evaluated 52 women between 65 and 80 years of age. During the first two years of the study, we decided to focus on the three (of five) subject groups that did not involve chemotherapy, i.e. the tamoxifen, ERT and control subjects. Thus, of the 52 subjects evaluated to date, 15 are women with breast cancer who are treated with tamoxifen (patient group), 18 are control subjects who are receiving estrogen replacement therapy (ERT) (positive control group), and 19 are control subjects who have never received ERT (negative control group). The following paragraphs describe the research accomplished for each approved Task.

Task 1. Preparation for Subject Recruitment and Data Collection, Months 1-2

During the first 2 months of the project, we held several meetings among the key investigators and research associates to discuss and implement subject recruitment. Screening checklists were prepared, which allowed the research associates to evaluate many of the inclusion and exclusion criteria in brief telephone interviews. Because potentially millions of healthy women (without breast cancer) in the US may soon receive tamoxifen for prevention of breast cancer, we decided to focus the initial phase of the study on the evaluation of the 3 subject groups that did not receive chemotherapy, i.e. breast cancer patients on tamoxifen only (tamoxifen group); healthy women receiving estrogen replacement therapy (ERT) (positive control group), and healthy women who did not receive ERT or tamoxifen (negative control group). This focused approach substantially accelerated our ability to answer the very important question whether tamoxifen has a negative impact on brain chemistry.

Task 2. Subject Recruitment and Data Collection, Months 3-32

During the initial project meetings, we decided to recruit women from several large ongoing studies at Harbor-UCLA Medical Center. These studies, including the WHI and WHIMS studies, involve very large cohorts of women. After obtaining approval from the local IRB and the local and overall PIs on these studies, women in the eligible age range were contacted by mail, and were asked to call a study coordinator if they were interested in participating in this study. Women who contacted the study coordinator were then asked if they would be willing to perform a brief telephone screen, which was designed to assess most of the inclusion and exclusion criteria. After passing the brief telephone screen, eligible subjects were scheduled for a visit at the Harbor-UCLA Clinical Research Center. During this visit, we first obtained verbal and written informed consent, followed by a more detailed evaluation, including routine blood tests, detailed medical history, neurological examination, general functioning evaluation (Karnofsky scale), structured interviews for depressive symptoms [Geriatric Depression Scale - Short Form – 10 items, to exclude subjects with excessive depression ($\geq$5 yes)] and anxiety/panic disorder (using Form A from Phase 2 of WHIMS: yes for anxiety questions and $\geq$4 out of 13 questions for panic disorders). Women who did not meet the study criteria were not allowed to participate any further.

Women who did meet the inclusion and exclusion criteria were then scheduled for an MRI / MRS scan and for neuropsychological testing (on two separate occasions). Altogether, our recruitment efforts have been very successful.

Task 3. Monitor Progress of Study, Months 3-32

As specified in the proposal, the Investigators, research assistants and research associates involved with the study met on an approximately regular basis. During these meetings, we monitored the progress of the subject recruitment, and discussed and resolved problems with the study. The recruitment of new subjects was slower in year 2, for several reasons. First, a major hardware upgrade took place on the MRI scanner at the Harbor-UCLA Imaging Center. This also will make it difficult to perform direct comparisons of proton MRS data acquired prior to and after the upgrade, and we will have to scan additional healthy control subjects to establish new normative values. Second, the original PI of the proposal, Thomas Ernst, Ph.D., moved to the Brookhaven National Laboratory, NY, in the past year. We would like to transfer the role of PI to Rowan Chlebowski, M.D., who previously was a Co-PI (a separate request was sent to the DOD regarding this issue). Dr. Ernst would maintain responsibility for all technical aspects of the study, including data analysis.

In addition, we have focused our efforts in 2 areas: First, we have finished the neuropsychological testing in all subjects, which lagged behind during year 1 of the study. Second, we have also performed a final analysis of the proton MRI data relating to the tamoxifen, ERT, and control groups (see next section). In the remainder of the study, we will focus on recruiting and studying elderly women who have been treated with (CMF) chemotherapy, and additional healthy control subjects (due to the scanner upgrade).

Task 4. Final Analysis and Publication, Months 33-36

As mentioned in the previous paragraph, we already have performed a final analysis of the data from 3 of the 5 groups (tamoxifen, ERT, and control); see next paragraph.

Preliminary results

We have performed a final analysis of the ^{1}H MRS findings in women who were treated with tamoxifen, women who received ERT, and women who received neither tamoxifen or ERT. We have presented these findings at two major meetings, the 2000 American Society of Clinical Oncology (ASCO) meeting and the 2000 meeting of the International Society of Magnetic Resonance in Medicine (ISMRM); see attachments. We have also prepared a manuscript describing our findings, which we submitted to the Journal of the National Cancer Institute (JNCI); see attachment for a copy of the manuscript. JNCI has tentatively accepted our paper pending revisions.

The manuscript reports on ^{1}H MRS in three brain regions (frontal white matter, basal ganglia, and hippocampus) in 76 elderly women, studied in three age-matched groups: 16 women receiving tamoxifen therapy, 27 women receiving estrogen replacement therapy (positive control group), and 33 women who had never received tamoxifen or estrogen (negative control group). The concentration of the putative glial marker myo-inositol [MI] was reduced in women receiving tamoxifen or estrogen, in comparison to women in the negative control group (overall group effect on ANOVA; $p=0.02$). The [MI] in the basal ganglia showed the most pronounced decreases (-16% in the tamoxifen group and -11% in the estrogen group), and was inversely related with the duration of tamoxifen treatment ($p=0.005$; Spearman correlation). No other significant metabolite abnormalities were observed. Because [MI] is increased in early brain injury and aging, the reduced [MI] in the tamoxifen and estrogen groups suggests that tamoxifen acts agonistic on brain estrogen receptors and has a similar neuroprotective effect as estrogen on the brain. Therefore, the findings of this study argue against our initial hypothesis that tamoxifen acts as an anti-estrogen in the brain. The lack of evidence for tamoxifen neurotoxicity on ^{1}H MRS, coupled with other accumulating evidence that tamoxifen is either non-harmful or even favorable to cognitive function, further reduce concerns about prescribing tamoxifen use for breast cancer risk reduction and other putative breast cancer risk.

(3) KEY RESEARCH ACCOMPLISHMENTS

- Created infrastructure to recruit study participants from ongoing large-scale studies at Harbor-UCLA Medical Center.
- Held monthly meetings to monitor the success of patient recruitment and resolve problems.
- Recruited 52 women in the correct age range and fulfilling all exclusion and inclusion criteria (15 in tamoxifen group; 18 ERT group; 19 negative control group).
- Finished neuropsychological testing in all subjects.
- Performed final statistical analyses of the ^{1}H MRS data for 3 groups (tamoxifen, estrogen, and negative control).

- Prepared and presented 2 abstracts at major scientific meetings.

- Prepared and submitted manuscript to JNCI (paper currently in revision).

(4) REPORTABLE OUTCOMES

Abstracts and presentations

- *Meeting of the American Society of Clinincal Oncolgy (ASCO):* R.T.Chlebowski, T.Ernst, L.Chang, D.Cooray, C.Salvador, Tamoxifen and Estrogen Effects on Brain Chemistry Determined by MR Spectroscopy (2001)

- *Meeting of the International Society of Magnetic Resonance in Medicine (ISMRM):* T.Ernst, L.Chang, K.Boone, D.Cooray, C.Salvador, R.Chlebowski, Effect of Tamoxifen Treatment on Brain Chemistry, # 551 (2001).

Manuscripts

- T.Ernst, L.Chang, D.Cooray, C.Salvador, J.Jovicich, I.Walot, K.Boone, R.Chlebowski, Estrogen and Tamoxifen Effect on Brain Metabolism in Elderly Women: A ^{1}H MR Spectroscopy Study, JNCI (in revision).

Funding

- We are in contact with Amgen, Inc., to perform a related study on the use of erythropoetin for improving CNS function.

(5) CONCLUSIONS

The concentration of the putative glial marker myo-inositol [MI] was reduced in women receiving tamoxifen or estrogen, in comparison to women in the negative control group. The concentrations of other brain metabolites, in particular N-Acetyl-aspartate (a putative neuronal marker) was normal in the ERT and tamoxifen groups. With regards to the administration of tamoxifen, this indicates that tamoxifen acts agonistic on brain estrogen receptors (i.e. is similar to estrogen), and has a similar neuroprotective effect as estrogen on the brain.

The lack of evidence for neurotoxicity on ^{1}H MRS in this study, coupled with the accumulating clinical evidence that tamoxifen is either non-harmful or even favorable to cognitive function, reduce concerns about prescribing tamoxifen use for breast cancer risk reduction and other putative breast cancer risk. This is extremely good news for women with breast cancer or those who have a high risk for developing breast cancer. It would be important to perform a larger longitudinal study with longer tamoxifen treatment periods to obtain more conclusive evidence that it is safe to utilize tamoxifen for breast cancer prevention, at least with regards to potential side effects on the brain.

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(7) APPENDICES

1. *Abstract, Meeting of the American Society of Clinincal Oncolgy (ASCO):* R.T.Chlebowski, T.Ernst, L.Chang, D.Cooray, C.Salvador, Tamoxifen and Estrogen Effects on Brain Chemistry Determined by MR Spectroscopy (2001)

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3. T.Ernst, L.Chang, D.Cooray, C.Salvador, J.Jovicich, I.Walot, K.Boone, R.Chlebowski, Estrogen and Tamoxifen Effect on Brain Metabolism in Elderly Women: A ^{1}H MR Spectroscopy Study, JNCI (in revision).

Appendix 1

TAMOXIFEN AND ESTROGEN EFFECTS ON BRAIN CHEMISTRY DETERMINED BY MR SPECTROSCOPY

R. T. Chlebowski, Harbor-UCLA Research and Education Institute, Torrance, CA, T. Ernst, L. Chang, Brookhaven National Laboratory, Upton, NY, D. Cooray, C. Salvador, Harbor-UCLA Research and Education Institute, Torrance, CA

Observational studies suggest cognitive function may be under hormonal influence. Since tamoxifen use for risk reduction can be considered in otherwise healthy women, we explored the effects of tamoxifen and estrogen on brain chemistry in elderly (≥65 years) women using localized ^{1}H magnetic resonance spectroscopy (MRS). MRI and localized ^{1}H MRS were performed in 76 women: 16 breast cancer patients (age 69.8±4.7 years) treated with tamoxifen (mean treatment period 4.4±1.7 years) who did not receive chemotherapy, 27 healthy women (age 71.4±4.0 years) treated with estrogen replacement therapy (ERT) mean treatment period 20.8±10.5 years, and 33 healthy women (age 71.7±4.5 years) who never took tamoxifen or estrogen. The cerebral metabolite concentrations of N-Acetyl compounds [NA], total creatine [CR], total choline [CHO] and myo-inositol [MI] were determined in the frontal white matter, basal ganglia, and hippocampus, using an established protocol for absolute quantitation. As seen below, the tamoxifen group had significantly lower [MI] in the basal ganglia (-13%; $p<0.05$) compared to controls who never received hormonal treatment; [MI] in the basal ganglia was negatively correlated with the tamoxifen treatment period ($p=0.005$; Spearman correlation coefficient = -0.72). Although there was a trend ($p=0.12$) for lower MI in the basal ganglia in the ERT group, no other significant metabolite differences were observed between the three groups in any of the 3 brain regions evaluated. Since normal aging and some cognitive dysfunctional status are associated with increase in the glial marker [MI], the reduction in [MI] in women receiving tamoxifen suggests tamoxifen may be associated with favorable modulation of regional "brain aging". To test this hypothesis, ongoing neuropsychological data will relate the ^{1}H MRS findings to clinical cognitive function.

Cerebral Metabolites (mmoles/kg) in Basal Ganglia of Women by Treatment Group

Group	[NA]	[CR]	[CHO]	[MI]
Tamox.	8.5±0.2	8.9±0.3	2.1±0.1	6.3±0.3*
Control	8.7±0.2	8.9±0.2	2.1±0.1	7.3±0.3
ERT	8.8±0.2	9.0±0.2	2.1±0.1	6.7±0.3
*Significance $p<0.05$ versus control				

Effect of Tamoxifen Treatment on Brain Chemistry

Thomas Ernst, Linda Chang, Kyle Boone, Dilrukshie Cooray, Corazon Salvador, Rowan Chlebowski
Brookhaven National Laboratory, NY, and Harbor-UCLA Medical Center, CA

Background: A substantial percentage of women with breast cancer after chemotherapy and hormonal modifying therapy (with tamoxifen) have reduced mental abilities compared to women who were not treated with chemotherapy and tamoxifen (1). Because tens of millions of healthy women who are at risk for breast cancer may soon obtain the anti-estrogen tamoxifen (and possibly other drugs) for preventative therapy, it is extremely important to know whether these drugs may cause injury to the brain (2).

Objective: To evaluate the potential effects of tamoxifen on brain chemistry, using localized ^{1}H magnetic resonance spectroscopy (MRS).

Design and Methods: MRI and localized ^{1}H MRS were performed in 16 women with breast cancer who were treated with tamoxifen (mean age 69.8±4.7 years; mean tamoxifen treatment period 4.4±1.7 years), and in 33 healthy women (mean age 71.7±4.5 years) who were never treated with tamoxifen or estrogen. The cerebral metabolite concentrations of N-Acetyl compounds [NA], total creatine [CR], total choline [CHO] and myo-inositol [MI], were determined in the frontal white matter, basal ganglia, and hippocampus, using a protocol for absolute quantitation (3, 4).

Table: Cerebral metabolite concentrations (in mmoles/kg).
Significance: * : $p < 0.05$

		[NA]	[CR]	[CHO]	[MI]
Basal ganglia	Tamox.	8.51 ±0.18	8.92 ±0.29	2.06 ±0.07	6.33 ±0.28*
	Control	8.66 ±0.17	8.92 ±0.18	2.14 ±0.06	7.27 ±0.28
Frontal WM	Tamox.	7.39 ±0.17	6.69 ±0.20	1.69 ±0.05	7.5 1±0.30
	Control	7.42 ±0.13	6.52 ±0.14	1.76 ±0.05	7.50 ±0.19
Hippo-campus	Tamox.	9.04 ±0.24	8.66 ±0.27	2.80 ±0.09	10.1 ±0.32
	Control	8.62 ±0.17	8.32 ±0.18	2.79 ±0.07	10.28 ±0.32

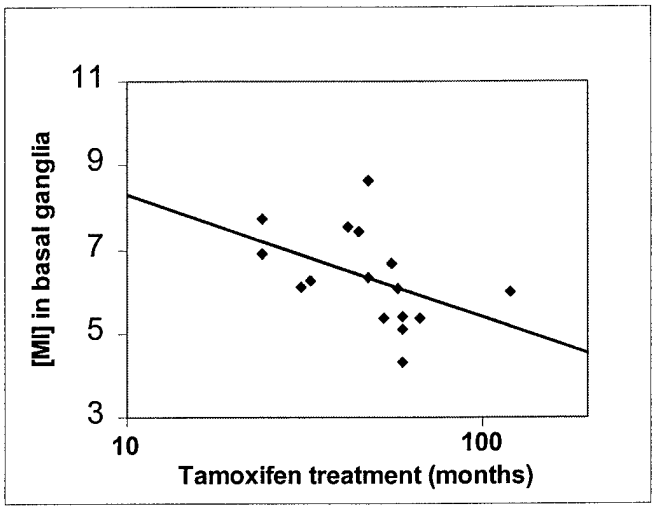

Figure: Relationship between the [MI] in the basal ganglia and the number of months patients were treated with tamoxifen.

Results: The tamoxifen group had lower [MI] in the basal ganglia (-13%; $p<0.05$) compared to women who were never treated with tamoxifen (see Table). The [MI] in the basal ganglia was negatively correlated with the tamoxifen treatment period ($p=0.005$; Spearman correlation coefficient = -0.72; see Figure). No other metabolite differences were observed between the two groups in any of the 3 brain regions evaluated.

Discussion: Our preliminary data indicate that women who have been using tamoxifen have reduced [MI] in the basal ganglia compared to age-matched women who did not receive tamoxifen. Since normal aging has been shown to be associated with increases in the glial marker [MI] (5), the reduced value in women on tamoxifen may be interpreted (with caution) as "slowing of brain aging", at least in the basal ganglia region. Alternatively, reduced [MI] may indicate abnormalities in the osmotic state of brain tissue (6). Future analyses of neuropsychological data will relate the ^{1}H MRS findings to cognitive function.

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Tamoxifen and Estrogen Effects on Brain Metabolism in Elderly Women Assessed by ^{1}H MR Spectroscopy

Thomas Ernst, Ph.D., Linda Chang, M.D., Dilrukshie Cooray, M.D., Corazon Salvador, M.D., Jorge Jovicich, Ph.D., Irwin Walot, M.D., Kyle Boone, Ph.D., Rowan Chlebowski, M.D.

Brookhaven National Laboratory, Medical Department **(T Ernst, L Chang)**

Harbor-UCLA Research and Education Institute, Departments of Medicine **(D Cooray, C Salvador, R Chlebowski),** Radiology **(J Jovicich, I Walot)** and Psychiatry **(K Boone),** Harbor-UCLA Medical Center, Torrance, CA 90502.

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Corresponding Author: Thomas Ernst, Medical Department, Building 490, Brookhaven National Laboratory, P.O.Box 5000, Upton, NY 11973-5000; e-mail: **ternst@bnl.gov**

Tel.(631) 344 8613; Fax (631) 344 2358.

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Key words: tamoxifen, estrogen, brain, metabolism, magnetic resonance spectroscopy.

Abstract

Background: Tamoxifen, an estrogen receptor agonist and antagonist used to treat breast cancer may also be used for breast cancer risk reduction after consideration of long term risks and benefits. To inform this process, the effects of tamoxifen on brain chemistry using sensitive biochemical markers associated with brain injury were assessed in elderly women using proton magnetic resonance spectroscopy (^{1}H MRS).

Methods: ^{1}H MRS was performed in three brain regions (frontal white matter, basal ganglia, and hippocampus) in 76 elderly women, studied in three age-matched groups: 16 women receiving tamoxifen therapy, 27 women receiving estrogen replacement therapy (positive control group), and 33 women who had never received tamoxifen or estrogen (negative control group).

Results: The concentration of the putative glial marker myo-inositol [MI] was reduced in women receiving tamoxifen or estrogen, in comparison to women in the negative control group (overall group effect on ANOVA; $p=0.02$). The [MI] in the basal ganglia showed the most pronounced decreases (-16% in the tamoxifen group and –11% in the estrogen group), and was inversely related with the duration of tamoxifen treatment ($p=0.005$; Spearman correlation). No other significant metabolite abnormalities were observed.

Conclusions: Because [MI] is increased in early brain injury and aging, the reduced [MI] in the tamoxifen and estrogen groups suggest that tamoxifen acts agonistic on brain estrogen receptors and has a similar neuroprotective effect as estrogen on the brain. These results, if confirmed in a longitudinal study, may reduce concerns regarding tamoxifen use for breast cancer risk reduction.

Introduction

Tamoxifen is an estrogen receptor agonist and antagonist widely used for treatment of breast cancer (*1*). Recent results suggest that tamoxifen reduces the risk of contracting breast cancer (*2,3*) and may be offered for this indication after consideration of long-term risks and benefits (*4-7*). While several studies have demonstrated positive effects of estrogen on metabolism and function of the brain (*8,9*), a preclinical study suggested that tamoxifen may act as an estrogen antagonist in the brain (*10*). Tamoxifen combined with chemotherapy in the clinic was associated with cognitive impairment (*11*). Such results led to the hypothesis that tamoxifen would negatively impact brain metabolism, especially in elderly women.

Therefore, a cross-sectional study in women receiving tamoxifen, women receiving hormonal replacement therapy (HRT) but no tamoxifen, and women who never received tamoxifen or HRT, was performed to evaluate brain metabolism using proton magnetic resonance spectroscopy (^{1}H MRS), a neuroimaging technique that provides sensitive biochemical markers associated with brain injury. Metabolites of particular interest include N-acetyl aspartate, a major constituent of the N-Acetyl (NA) peak and a neuronal marker that reflects neuronal density and integrity, and myo-inositol (MI), a tentative glial marker that reflects glial content or activity. Since numerous MRS studies in various neurological disorders have implicated glial proliferation (in response to brain injury) with increased cerebral MI (*12,13*), we hypothesized that a presumptive estrogen-antagonistic effect will be associated with increased cerebral MI, whereas estrogen agonistic effect will be associated with reduced cerebral MI, reflecting a potential protective role in the brain.

Methods

Three groups of age-matched women (total n=76) between the ages 65-80 years were recruited from the local community through advertisements and from solicitations through the mail. Verbal and written informed consent was obtained from each woman, according to the Institutional Review Board approved procedures and in accord with an assurance filed with and approved by the U.S. Department of Health and Human Services. All subjects fulfilled the following inclusion criteria: The tamoxifen-only group (n=16) included women with diagnosed breast cancer (originally localized and resected) who had been receiving tamoxifen (20 mg/day) for $\geq$ 2 years (range: 2-10 years; mean: 4.4±1.7 years), but never received any systemic chemotherapy or estrogen as HRT. The positive control group (n=27) consisted of healthy women without a history of breast cancer, who had been receiving HRT for $\geq$ 2 years (mean: 20.8±10.5 years). HRT consisted of oral conjugated equine estrogen (Premarin 0.625 mg/day, n=25; Premarin 1.25 mg/day, n=1; and Estropipate 0.75 mg/day, n=1); in addition, 3 women who were receiving Premarin (0.625mg/day) also required intermittent medroxyprogesterone (Provera 10mg/day). The negative control group (n=33) consisted of healthy women without a history of breast cancer who were never treated with tamoxifen, chemotherapy, or HRT. In addition, all subjects were evaluated by a physician at the General Clinical Research Center to ensure that they did not meet the following exclusion criteria: recurrent breast cancer, psychiatric disorders, chronic medical or neurological illnesses that might impact cognition (e.g., uncontrolled hypertension, abnormal thyroid function, diabetes, strokes, Alzheimer's or Parkinson's disease), history of head trauma with loss of consciousness for more than one hour, or any contraindication for the imaging studies. The clinical evaluations included a history, physical and neurological examination, blood pressure assessments, an electrocardiogram, a battery of

comprehensive screening blood tests [complete blood count, chemistry panel, thyroid function tests, rapid plasma reagin (RPR), vitamin B12 and folate levels], and urinalysis. Each subject also completed a standardized cognitive screening form [modified Mini-Mental State Examination (MMSE) (*14*)] that was used in the multi-center Women's Health Initiative Memory Study (WHIMS).

MRI scans were performed on a 1.5 Tesla GE scanner. Following a sagittal T1-weighted localizer [echo time / relaxation time (TE/TR) = 11/500ms, 5-mm thickness, 1-mm gap], an axial fast spin echo sequence (TE1/TE2/TR = 17/102/4000 ms, 5-mm thickness, no gap), and an axial fluid-attenuated inversion recovery sequence (TE/TI/TR = 142/2600/11000 ms) were performed. Localized ^{1}H MRS was obtained in three brain regions: the frontal white matter, basal ganglia, and left hippocampal region. MRS voxels (3-5 cc) were carefully placed to ensure the inclusion of the same anatomical structures in each subject. MRS data were acquired using a double spin echo sequence, or point resolved spectroscopy (PRESS), which was optimized for acquisition of ^{1}H MR spectra from the frontal lobe (*15,16*). The acquisition parameters were TE/TR = 30/3000 ms; 64 averages were acquired. Cerebral metabolite concentrations of NA, MI, total creatine (CR) and choline-containing compounds (CHO), corrected for the presence of cerebrospinal fluid in each voxel, were determined using methods previously described (*17*).

Statistical analyses were performed in StatView (version 5.0, SAS Institute, Cary, NC). A mixed two-way analysis of variance (ANOVA) was performed to determine the significance of differences in cerebral metabolite concentrations among the three brain regions (within-variable) and the three subject groups (between-variable). For variables that showed significance on ANOVA, Student's t-tests were additionally performed to determine the significance of

differences between individual groups, separately for each brain region. Spearman correlations were performed to test for possible relationships between metabolite concentrations and duration of treatment with tamoxifen or estrogen. A type I error probability ≤ 0.05 (two-tailed) was used to determine statistical significance.

Results

The age of the women in the three groups was tightly matched and not significantly different among the groups (p=0.52), with mean ages of 71.8±4.1 years for the control subjects (mean ± standard deviation), 71.5±4.1 years for the HRT group, and 70.4±4.7 years for the tamoxifen group. No difference in education was found between the three groups (control: 14.1 years; HRT: 14.8 years; tamoxifen: 13.2 years; p=0.15). The screening cognitive assessment showed no cognitive deficits or group difference in these women (tamoxifen: 95.9 ± 3.5; ERT: 95.4 ± 4.5; control: 95.2 ± 4.9; maximum = 100). Similarly, none of the women showed abnormalities on neurological examination, except for 19 women who had mild essential tremor (HRT group: n = 6 or 22%; tamoxifen group: n = 5 or 31%; negative control group: n = 8 or 24%). Structural MRI scans showed no major structural abnormalities (i.e. cortical infarcts, tumors or vascular malformations) in any subject. However, nearly half of all women (49%) showed small or moderate white matter hyperintensities in the periventricular regions; no group differences were observed with respect to these lesions. Additionally, silent lacunar infarcts (small lesions showing hyperintensity on T2-weighted MRI and hypointensity on T1-weighted images) were observed in three women in the HRT group, but none in the other two groups.

The ANOVA demonstrated a significant group effect ($p=0.02$) and regional effect ($p<0.001$) on the MI concentration ([MI]), but no interaction between the two variables. There was a significant overall decrease of [MI] both in the tamoxifen ($p=0.01$) and the estrogen group ($p=0.03$) compared to the control subjects. This decrease was most pronounced in the basal ganglia, where [MI] was significantly reduced in the tamoxifen group (-16%; $p=0.004$; significant after Bonferroni adjustment for 9 comparisons; see Table and Figure) and showed a trend for reduction in the estrogen group (-11%; $p=0.06$). Furthermore, the duration of tamoxifen treatment showed an inverse relationship with [MI] in the basal ganglia (Spearman correlation; $p=0.005$; rho = -0.72; Figure 1) and in the hippocampus ($p=0.04$; rho = -0.50). There were no significant group, regional, or interaction effects for the other three metabolites, in particular not for NA, the neuronal marker.

Discussion

Our initial hypothesis that an estrogen-antagonist effect of tamoxifen in the CNS would lead to increased glial response with an associated increased [MI], as a potential marker of early neurologic injury, proved incorrect. In contrast, [MI] was significantly decreased in both the tamoxifen and estrogen groups compared to the control group without hormonal therapy. Since normal aging is associated with increases in the glial marker [MI] (*18*), the reduced [MI] in women receiving tamoxifen or estrogen suggests that both of these hormone-modifying therapies may be neuroprotective and associated with favorable modulation of "brain aging".

Several recent reports support our findings and the conclusion that both tamoxifen and estrogen use may be neuroprotective and growth-promoting in the brain. In preclinical studies, estrogen has been shown to be neuroprotective (*19,20*), possibly by blocking oxidative stress-

induced neuronal death (*21*). Both estrogen and tamoxifen also were protective against glutamate-mediated cytotoxicity in glial cells and stimulated cell differentiation (*22*). Induction of aromatase, the enzyme that produces estrogen *de novo* in astrocytes, is thought to be part of the glial repair response to brain injury (*23*). Similarly, estrogen receptors were expressed in reactive astrocytes in response to injury in the primate brain (*24*). Finally, both estrogen and tamoxifen increased synaptic density in ovariectomized rats (*25*). These findings all support a neuroprotective or repair role of estrogen. With normal aging, increased astrocyte activity has been documented with glial fibrillary acidic protein (a histological glial marker) (*26*), which further supports our conclusion that decreased [MI] (the glial marker observable with MRS) in women who received estrogen or tamoxifen may be associated with slowing of the aging process. Furthermore, estrogen receptors are located in the basal forebrain, the hypothalamus area and the basal ganglia (*27*), which is the region that showed the most pronounced decrease in [MI].

In a clinical observation, a large scale, retrospective cross- sectional study of nursing home residents using the New York State Medical Data System (NYS-MDS) to evaluate 6,925 women over 65 years of age in matched sets (each set consisted of one woman with tamoxifen and four women without tamoxifen use) found that women receiving tamoxifen were less likely to have a diagnosis of Alzheimer's disease, were significantly ($p<0.01$) more independent in activities of daily living (bed mobility, eating, toileting, personal hygiene, dressing, transferring, locomotion), and had better cognitive skills for daily decision-making (*28*). Two studies in relatively younger tamoxifen users did not find a positive effect of tamoxifen on cognition. The first is from the health related quality of life component of the National Surgical Adjuvant Breast and Bowel

Project Prevention Trial, which reported baseline and 36 months data on 11,064 women (average 58 years old), using screening questionnaires (Center for Epidemiological Studies-Depression Scale (CES-D) and the Medical Outcomes Study 36-Item Short Form Health Status Survey (MOS SF-36)) (*29*). Women treated with tamoxifen and placebo had similar depressive symptoms and cognitive function. Contrary to this study, the elderly nursing home women in the aforementioned NYS-MDS study were 42% more likely to have a diagnosis of depression if they received tamoxifen. Another study employed the mailing of a follow-up questionnaire to assess cognitive function (clock drawing, copying a box and narrative writing to describe a picture) in 1,163 women with breast cancer aged 57-75 years, and found no decreased cognitive function in those who had used tamoxifen for the standard term (4-5 years), although the women who were current users complained about memory problems more than the non-users (*30*). However, potential confounds in these studies, such as the diagnosis of cancer or other chronic medical illnesses as causes for increased depression (in the NYS-MDS study) and the higher frequency of follow-up visits to doctors in current tamoxifen users (in the Paganini-Hill and Clark report) accounting for increased memory complaints, need to be controlled for in future prospective studies.

The vast majority of breast cancer adjuvant trials were not designed to prospectively collect information regarding effects of tamoxifen on cognition. Nonetheless, four studies have evaluated cognitive function in women following adjuvant chemotherapy (*31,32*) or chemohormonal therapy (*11,33*). In all reports conventional chemotherapy, whether alone (*31*) or combined with tamoxifen (*11*), and especially high dose chemotherapy (*11,32*) was associated with increased cognitive dysfunction. In only one report (*33*) was tamoxifen use considered

separately from chemotherapy. In this setting, tamoxifen use had no influence on patient's self reports of cognitive function.

Taken together, the findings from studies in the literature regarding tamoxifen influence on cognition are consistent with the normal screening cognitive assessments seen for women on tamoxifen in the current report. However, these large surveys and our screening assessments of cognition all employed relatively simple scales with recognized sensitivity limitations; therefore more detailed neuropsychological tests are necessary to further determine whether tamoxifen use is only non-harmful or whether it might have a favorable effect on cognitive function. Age may be another important variable, since the NYS-MDS study included only elderly women 65 years or older; it is possible that tamoxifen's neuroprotective effect is most evident in older patients who would have less cognitive reserve.

In contrast to the subject surveys, in which responses and/or performance might be confounded by such factors as effort or depression, metabolite concentrations, [NA] and [MI] measured on MRS may serve as objective surrogate markers to evaluate the integrity of neuronal and glial function, respectively. This study found no difference in [NA] in women who received tamoxifen compared to the two control groups. This suggests a lack of neuronal injury or loss in the tamoxifen users, and, together with the similar effect of tamoxifen and estrogen on [MI], argues against an anti-estrogen activity of tamoxifen in the brain.

In conclusion, the decreased concentration of the glial marker MI in women taking tamoxifen or estrogen suggests both drugs may be neuroprotective and have favorable modulatory action on aging. The lack of evidence for neurotoxicity on ^{1}H MRS, coupled with the accumulating evidence that tamoxifen is either non-harmful or even favorable to cognitive function, further reduce concerns about prescribing tamoxifen use for breast cancer risk reduction and other putative breast cancer risk. Future prospective, longitudinal studies involving MRS

and detailed neuropsychological testing of tamoxifen and other selective estrogen receptor modulators, and aromatase inhibitors under evaluation for breast cancer risk reduction, are needed to document their long term effects on cognitive function.

Table: **Cerebral metabolite concentrations (in mmoles/kg ± standard error).**

		[NA]	[CR]	[CHO]	[MI]†
Basal ganglia	Tamoxifen	8.51 ±0.18	8.92 ±0.29	2.06 ±0.07	6.33 ±0.28*
	Control	8.66 ±0.17	8.92 ±0.18	2.14 ±0.06	7.57 ±0.27
	HRT	8.75 ±0.15	8.97 ±0.19	2.13 ±0.07	6.73 ±0.33
Frontal WM	Tamoxifen	7.39 ±0.17	6.69 ±0.20	1.69 ±0.05	7.51±0.30
	Control	7.42 ±0.13	6.52 ±0.14	1.76 ±0.05	7.57 ±0.19
	HRT	7.49 ±0.14	6.37 ±0.13	1.67 ±0.06	7.28 ±0.22
Hippocampus	Tamoxifen	9.04 ±0.24	8.66 ±0.27	2.80 ±0.09	10.10 ±0.32
	Control	8.62 ±0.17	8.32 ±0.18	2.79 ±0.07	10.60 ±0.24
	HRT	8.69 ±0.21	8.51 ±0.19	2.74 ±0.08	9.82 ±0.25

Statistical significance: * : $p \leq 0.004$ (Tamoxifen vs. negative control subjects; significant after correction for multiple (n=6) comparisons); †: $p < 0.02$ (group effect on ANOVA)

Figure legend

Figure: Representative ^{1}H MR spectra from the basal ganglia region of three different subjects. Myo-inositol (MI) is reduced in the women receiving HRT or tamoxifen. The voxel location in the basal ganglia is indicated in the axial MRI (top right). The graph shows the dependence of [MI] in the basal ganglia on the duration of tamoxifen treatment (logarithmic scale).

Abbreviations: NA = N-Acetyl resonance; CR = creatine plus phosphocreatine resonance; CHO = choline resonance.

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